UNIVERSITY OF GONDAR

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

Simultaneous Determination of Caffeine and Paracetamol Using Activated Glassy Carbon Electrode

By: Tilahun Yai

Advisor: Dereje Yenealem (MSc)

June, 2017

Gonder, Ethiopia

By: Tilahun Yai

Simultaneous Determination of Caffeine and Paracetamol Using Activated Glassy Carbon Electrode

Advisor: Dereje Yenealem (MSc)

Thesis Paper Submitted to the Department of Chemistry College of Computational and Natural Science, University of Gondar in Partial Fulfilment of the Requirements for the Degree of Masters of Science in Chemistry (Physical Chemistry)

UNIVERSITY OF GONDAR

COLLEGE OF COMPUTATIONAL AND NATURAL SCIENCE

DEPARTMENT OF CHEMISTRY

Simultaneous Determination of Caffeine and Paracetamol Using Activated Glassy Carbon Electrode

Thesis Paper Submitted to the Department of Chemistry, College of Natural and Computational Science, University of Gondar in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Chemistry

Approved by

1**. Advisor**: Dereje Yenealem (M.Sc.) **Date** _____ **Signature** _____

Examining Board

Name		**Signature**	**Date**
1. Muluken Aklilu (Ph.D)	(Examiner)	_____	_____
2. Molla Tefera (Ph.D)	(Examiner)	_____	_____
3. _____	(Chairman)	_____	_____

JUNE, 2017

TABLE OF CONTENTS

Contents **Pages**

Acknowledgments

I would like to express my deepest gratitude to my research advisor Mr Dereje Yenealem for his advice while conducting this work.

Above all, I would like to thanks Dr. Meareg Amare for his unreserved guidance, technical and moral support and encouragement during the entire project work.

I would like to acknowledge the Department of Chemistry, BDU, for giving permission to conduct this work in their university and for their unlimited support through all the material used for this project.

I would also like to thanks department of Chemistry, UoG for their support by giving different chemicals and materials needed for this work

Lastly, I would like to thanks Ethiopian minister of education for giving this opportunity to attend my MSc study at UoG and their financial support to conduct this work.

List of Abbreviations

4α-Cu^{II}TAPc SAM - Self-Assembled Monolayer of Non-Peripheral Amine Substituted Copper (II) Phthalocyanine

AAAH- Aromatic Amino Acid Hydroxylases

AADC or AAAD- Aromatic L-Amino Acid Decarboxylase

AC- Activated Carbon

AGCE - Activated Glassy Carbon Electrode

APF – Addis Pharmaceutical factory

CAF- Caffeine

CV- Cyclic Voltammetry

DLC: VAMWCNT - Vertically Aligned Multi-Walled Carbon Nanotubes and Diamond-like Carbon Films

DME- Dropping Mercury Electrode

DOPA- L-3, 4-Dihydroxyphenylalanine

DPV- Differential Pulse Voltammetry

E_{pa}- Anodic Peak Potential

E_{pc}- Cathodic Peak Potential

ΔE_p – Change in Potential

ET- Electron Transfer

GCE- Glassy Carbon Electrode

HPLC- High Performance Liquid Chromatography

I_{pa} - Anodic peak Current

I_{pc}- Cathodic Peak Current

LOD – Limit of Detection

LOQ – Limit of Quantification

NAPQI - N-Acetyl-P-Benzoquinone Imine

PA- Paracetamol

Poly(AHNSA) - Poly(4-amino-3-Hydroxynaphthalene Sulfonic Acid)

PBS - Phosphate Buffer Solution

R^2 –Regeression Equation

SWV- Square Wave Voltammetry

υ – Scan Rate

List of Figures **pages**

List of Tables **pages**

List of Schemes **pages**

Abstract

In this study the electrochemical behaviour of AGCE was compared with bare GCE for the determination of CAF and PA using CV and SWV. The result of CV was shown that, the PA exihibit only oxidation peak current at GCE, which reveals irreversible properties of PA at GCE. Intense oxidation and reduction peak of PA with oxidation peak potential shift to negative potential was observed at AGCE. Even though, its response current was decreased, the oxidation peak potential of CAF was shift to more negative side at AGCE in comparison to GCE. This was due to good catalytic effect of AGCE toward the redox reaction of studied samples. SWV was employed for simultaneous determination of CAF and PA.The relationship between peak current and concentration of PA was studied by varying its concentration from (10 – 180 μM) at constant concentration of 0.5 mM CAF and result in linear over studied range with correlation coefficient of (R^2 = 0.990). Similarly, CAF was studied by varying its concentration from (100 – 950 μM) at constant concentration of 10 μM PA and it was also linear with its response over studied range with correlation coefficient of (R^2 = 0.991).The detection and quantification limit (LOD and LOQ) were 2.55 μM, 0.36 μM and LOQ 8.49 μM, 1.21 μM for PA and CAF, respectively. The validity of the proposed method was checked by using commercial tablet which contain different amount of CAF and PA and % recoveries were calculated (Recovery = 94.54% and 96.66% for CAF and PA respectively). Uric acid was used as interference to study its effect on simultaneous determination of CAF and PA and the result was shown that uric acid had effect on peak potential and highly minimize the peak current of both CAF and PA.

Key words*: Caffeine, Paracetamol, Glassy Carbon Electrode, Activated Glassy Carbon Electrode, Limit of Detection , Limit of Quantification, Validity and Interference*

1. Introduction

PA is an acylated aromatic amide that was firstly introduced in medicine by Von Mering in 1893 as an antipyretic/analgesic. It has low toxicity when used at recommended doses. The drug is of worldwide application for the relief of postoperative pain as well as mild to moderate pain associated with headache, backache and arthritis [1, 2, 3]. PA is also known as an anticancer drug that tends to supplant aspirin for patients who are allergic to aspirin. In the case of drug overdose, accumulation of toxic products leads to severe kidney and liver problems. Nausea, vomiting, perspiration, and tiredness are the other reported mal-effects of PA overdose [4].

CAF is alkaloid of nitrogen containing organic metabolite produced by plants, the plants that produce this alkaloid make their leave unattractive to eating by insects and higher animals. The awfully known toxic compounds morphine, quinine, cocaine and codeine belong to this group of compounds [5]. A dose of 200-500 mg of caffeine is generally sufficient for a mild stimulation. Overdose of caffeine may result in hypertension, vomiting and cardiac arrest [6].

CAF is metabolized in the liver into three primary metabolites: paraxanthine (84%), theobromine (12%), and theophylline (4%). CAF is completely absorbed by the stomach and small intestine within 45 minutes of ingestion. After ingestion it is distributed throughout all tissues of the body and is eliminated by first-order kinetics [7].

The half-life of CAF, the time required for the body to eliminate one-half of the total amount of CAF consumed at a given time varies widely among individuals according to such factors as age, liver function, pregnancy, some concurrent medications, and the level of enzymes in the liver needed for CAF metabolism. In healthy adults, caffeine's half-life is approximately 3-4 hours. In women taking oral contraceptives this is increased to 5-10 hours, and in pregnant women the half-life is roughly 9-11 hours. CAF can accumulate in individuals with severe liver disease when its half-life can increase to 96 hours. In infants and young children, the half-life may be longer than in adults; half-life in a newborn baby may be as long as 30 hours. Other factors such as smoking can shorten CAF half-life time [8, 9].

The liver, kidney and intestine are the major organs implicated in the metabolism of PA. After a therapeutic dose, PA is mostly converted to pharmacologically inactive glucuronide (PA-gluc, 52-

57% of urinary metabolites) and sulfate (PA sulfate, 30-44%) conjugates, with a minor fraction being oxidized to a reactive metabolite *N*-acetyl-*p*-benzoquinone imine NAPQI (5-10%). Less than 5% of PA is excreted unchanged. PA disposition involves a complex inter-organ transport of metabolites between the liver, kidney and intestine, through bile and the blood stream, to be ultimately eliminated in urine. The kidney is the main site of the disposition of PA sulfate, either through direct excretion or through further biotransformation followed by renal excretion. Although most of NAPQI is formed in the liver, the kidney also metabolizes PA to the toxic metabolite and releases cysteine conjugate of PA into the bile and blood for further elimination in urine [10].

Different methods have been employed for the determination of CAF and PA individually or simultaneously which include thin layer chromatography, spectrophotometry, liquid chromatography, micellar electrokinetic capillary chromatography, chemiluminescence, high-performance liquid chromatography (HPLC), solid-phase molecular fluorescence and spectrofluorimetry. These methods have some drawbacks such as involving tedious procedures and more expensive. To overcome these drawbacks, consequently the development of inexpensive, simple, sensitive, and reliable electroanalytical methods is of considerable importance [2].

Currently, electroanalytical methods has wide variety application in single and simultaneous determination of drug concentration in a sample by using bare and modified electrodes. In spite of this, electroanalytical methods have rarely been used for the analysis of CAF, mainly because its oxidation occurs at a very positive potential, which overlaps with that for the discharge of the electrolytic solution. Bare electrode has poor sensitivity and selectivity towards the determination of substance in unknown sample. In order to improve these low performance, electrodes were modified by employing various materials, such as nanoparticles [11], polymers [12], metal oxides [13], and integrated nanomaterials [14]. Considerable reports were available in the literature for the determination of CAF and PA by using different chemically modified electrode, such as, electrochemical determination of CAF in the presence of PA using a self-assembled monolayer of non- peripheral amine substituted copper(II) phthalocyanine [6], simultaneous voltammetric determination of PA and CAF in pharmaceutical formulations using a boron-doped diamond electrode [15], Voltammetric determination of PA, tramadol and CAF using poly(Nile blue) modified glassy carbon electrode, Simultaneous electrochemical determination

of PA, CAF and ascorbic acid using a new electrochemical sensor based on CuO-graphene nanocomposites [16], Simultaneous Voltammetric Determination of PA, Codeine and CAF on Diamond-like Carbon Porous Electrodes [17]. Although these modified electrodes successfully determined the concentrations of both caffeine and paracetamol, the fabrication of an electrochemical sensor with high sensitivity and selectivity is still one of the challenging tasks for the researchers.

A great variety of solid electrodes have been employed in different voltammetric techniques over the years. Of the many different solid materials that can be used as working electrodes, the most commons are; carbon, platinum, and gold. Carbon-based electrodes usually have a wider application than the other solid electrodes because of their broad potential window, low background current, rich surface chemistry, chemical inertness, low cost and suitability for various sensing and detection applications [17].

The most commonly used carbon-based electrode in the analytical laboratory is glassy carbon electrode. Glassy carbon is also known as vitreous carbon. Glassy carbon or vitreous carbon, as the name implies, is a form of glass-like carbon which combines some of the properties of glass with some of those of normal industrial carbons. It has been very popular because of its excellent electrical and mechanical properties, wide working potential range, extreme chemical inertness and relatively reproducible performance. Additional activation steps, such as electrochemical, chemical, heat, or laser treatments have also been used to enhance the performance. Among these, the electrochemical activation is considered a preferable and rapid in situ pretreatment technique, which consume less time and easy to work with. In this study, a very simple and cheap pretreatment bare glassy carbon electrode (GCE) was used to determine CAF and PA simultaneously.

2. Literature Review

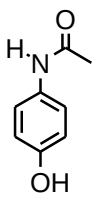
2.1. Electrochemical Activation and Chemical Modification of Electrode

Chemical modification and surface treatment of a solid electrode is method to extensively improve the electrochemical performance of the electrode. Especially, electrochemical pretreatment is used for cleaning and activating surface of electrode. The surfaces of the metal and carbon electrodes can be oxidized, and thus various kinds of oxygenous groups, such as phenolic, quinoidal, and carboxyl functionalities, can be added on the surfaces[18, 19, 20].

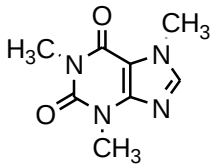
The goals of chemical modification are to acceleration electron transfer reactions at the electrode surface, changing transport properties to the electrode surface, creating selective membrane permeation, and interferent exclusion. However, its responses are depend on the preparation method of the chemically modified electrode, and in particular, factors such as surface coverage, film composition and morphology. Electropolymerisation, chemisorptions, covalent (chemical) attachment (silanisation or direct bonding), sol-gel encapsulation, physical adsorption, and the Langmuir-Blodgett method (creating highly ordered monolayer films) can be employed to attach modifiers to solid electrode surfaces [21].

2.2. Caffeine and Paracetamol

CAF (1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione), is a xanthine derivative naturally present in cocoa, coffee and tea and is added to many beverages. The presence of CAF in coffee, beverages and tea acts as a diuretic and stimulant to the central nervous and cardiovascular systems. CAF are used in analgesic pharmaceutical formulations. PA (N- acetyl – P- aminophenol) is a widely used antipyretic drug which serves as an effective and safe analgesic agent in therapeutic practices [22, 23, 24] (**scheme 1**).



Paracetamol

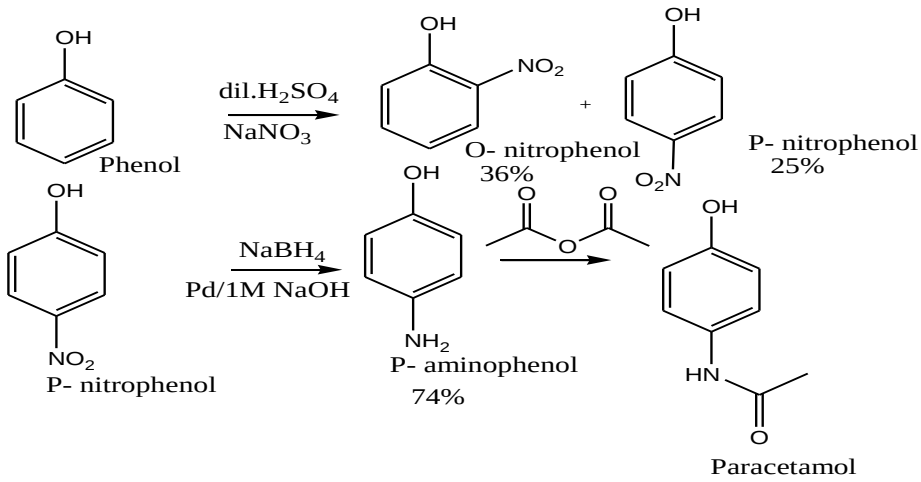


Caffeine

Scheme 1. Chemical structure of paracetamol and caffeine

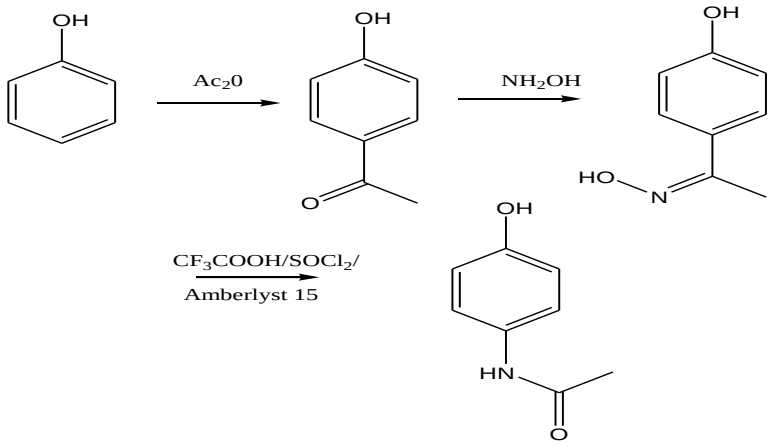
2.2.1. Synthesis of Caffeine and Paracetamol

The original method for production of PA involves the nitration of phenol with sodium nitrate gives a mixture of two isomers, from which the wanted 4-nitrophenol (bp 279 °C) can easily be separated by steam distillation. In this electrophilic aromatic substitution reaction, phenol's oxygen is strongly activating, thus the reaction requires only mild conditions as compared to nitration of benzene itself (**scheme 2**). The nitro group is then reduced to an amine, giving 4-aminophenol. Finally, the amine is acetylated with acetic anhydride. Industrially direct hydrogenation is used, but in the laboratory scale sodium borohydride serves [25, 26].



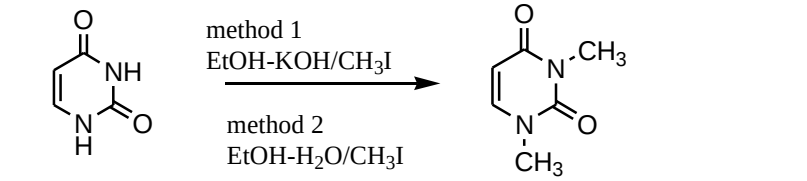
Scheme 2. Synthesis of PA from phenol in the laboratory

An alternative industrial synthesis developed by Hoechst–Celanese involves direct acylation of phenol with acetic anhydride catalyzed by hydrogen fluoride, conversion of the ketone to a ketoxime with hydroxylamine, followed by the acid-catalyzed Beckmann rearrangement to give the amide (PA) (**scheme 3**).



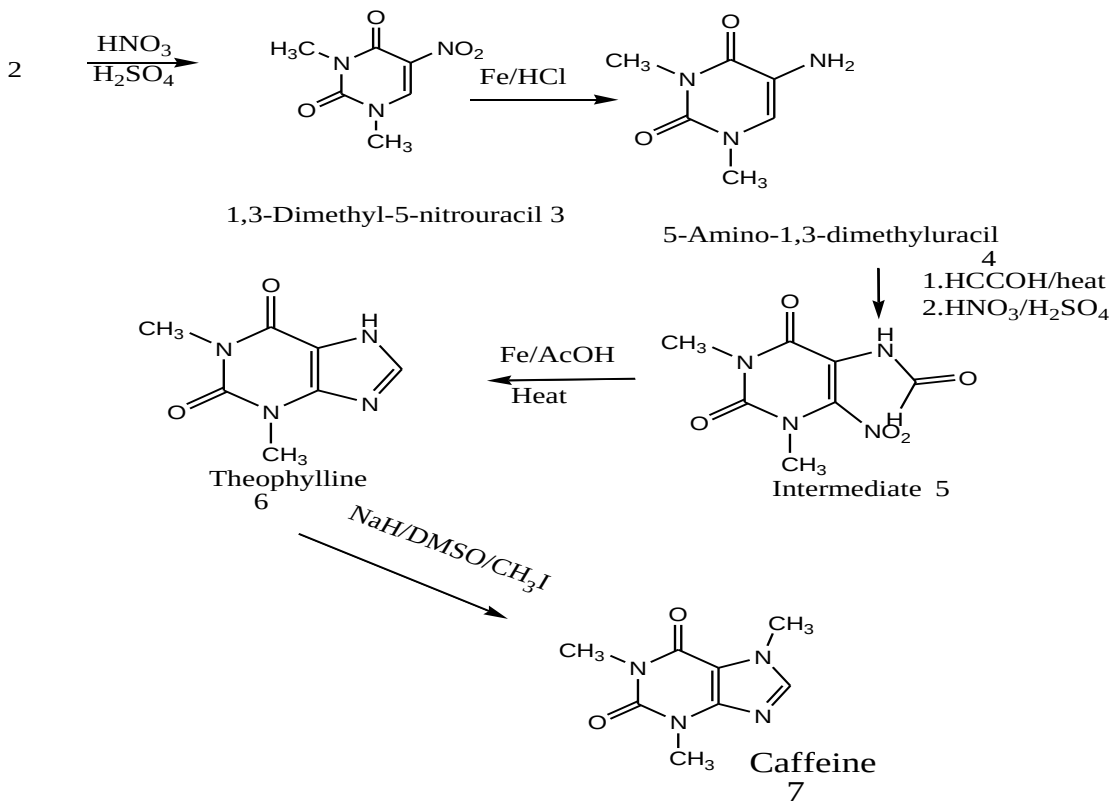
Scheme 3. Alternative industrial synthesis of PA

Simple synthesis of CAF 7 from uracil 1 by N-methylation, nitration, reduction, and cyclization reactions (**scheme 4**). The reaction of uracil with excess of methyl iodide with potassium hydroxide and ethanol (Method 1) or in an aqueous ethanol medium (Method 2) resulted in a very poor yield of 1,3-dimethyluracil 2. On the other hand, the presence of a strong base such as sodium hydride in dimethylsulfoxide, a nonaqueous solvent facilitates the methylation tremendously (Method 3). This results in an increased yield of 60% of 1,3-dimethyluracil 2, compared to Method 1 (10%) or Method 2 (20%).



Uracil 1 method 3 NaH/DMSO/CH_3I 1,3 - dimethyluracil 2

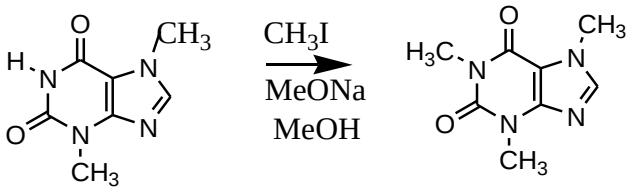
Conventional nitration using a nitrating mixture of fuming nitric acid and sulfuric acid produces 64% yield of 3. The reaction was rapid and isolation of the compound was very easy. The nitro group in compound 3 was reduced using iron and hydrochloric acid in tetrahydrofuran (THF) to produce 5-amino-1,3-dimethyluracil 4.



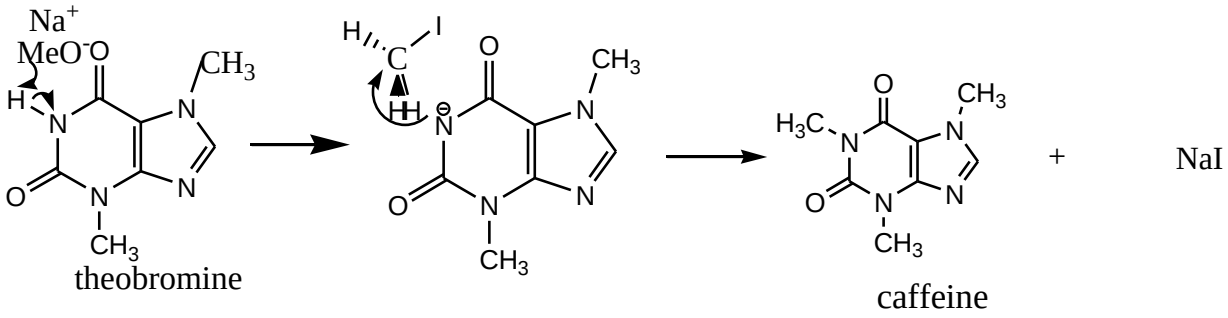
Scheme 4. Laboratory Synthesis of CAF from uracil

Heating compound 4 with formic acid generated in situ, the formamide derivative of compound 4 (not shown). Nitration of the formamide derivative introduces a nitro group in position 6 of uracil to produce compound 5 as a light yellow colored solid. Reduction and intramolecular heterocyclization of compound 5 was simultaneously carried out with iron and acetic acid to produce theophylline (75%) 6. Theophylline was purified by flash chromatography on silicagel using ethyl acetate as the solvent. The final step of the synthesis involves N-methylation at position 7 of theophylline using method 3 to produce CAF [27].

CAF is also synthesized in the laboratory by methylation of theobromine with dimethyl sulphate. The N-alkylation of theobromine using methyl iodide in methanolic sodium methoxide solution allows an efficient and rapid synthesis of CAF. The reaction goes to completion at room temperature in 90 min, or in 40 min at 60 °C, and the product is free of contaminants giving 90% of yield [28].



Scheme 5. Caffeine synthesis from theobromine



Scheme 6. Reaction mechanisms. N-methylation of theobromine occurs via S_N2

2.3. Electrochemical Techniques

Electrochemical techniques are analytical techniques that use a measurement of potential, charge, or current to determine an analyte concentration or to characterize an analyte chemical reactivity. Electrochemical measurements are made in an electrochemical cell consisting of two or more electrodes and the electronic circuitry for controlling and measuring the current and the potential. Because we cannot simultaneously control the current and the potential, there are only three basic experimental designs in electrochemical cell: (i) measuring the potential when the current is zero, (ii) measuring the potential while controlling the current, and (iii) measuring the current while controlling the potential. Electrochemical methods are classified as bulk and interfacial methods. Bulk methods measure properties of the whole solution. For example, conductometry measures the conductivity of a solution, which is proportional to the total concentration of dissolved ions. Interfacial methods measure signals that are functions of phenomena occurring at the interface between an electrode and the solution in contact with the electrode [29].

Voltammetry is one type of an interfacial electroanalytical technique based on the measurement of current flowing through an electrode dipped in solution containing electroactive compounds

while a potential is imposed upon it. It is typically performed using a three electrode potentiostat, which accurately controls the applied potential. The redox reaction takes places at working electrode, because it is where the reaction of interest is taking place. The working electrode may be solid (platinum, gold, silver), drop of mercury, carbon etc. If the working electrode is drop of mercury the technique is called polarography. The second electrode is a reference electrode, which maintains a constant potential throughout the experiments and the third electrode the counter (auxiliary) electrode, which complete the electrical circuit. The common characteristic of all voltammetric technique is that they involve the application of a potential (E) to an electrode and monitoring of the resulting current (I) flowing through electrochemical cell [30].

2.3.1. Cyclic Voltammetry

Cyclic voltammetry (CV) has become an important and widely used electroanalytical technique in many areas of chemistry. It is rarely used for quantitative determinations, but it is widely used for the study of redox processes, for understanding reaction intermediates, and for obtaining stability of reaction products. This technique is based on varying the applied potential at a working electrode in both forward and reverse directions (at some scan rate) while monitoring the current. For example, as shown in **Figure** 1 the initial scan could be in the negative direction to the switching potential. At that point the scan would be reversed and run in the positive direction. Depending on the analysis, one full cycle, a partial cycle, or a series of cycles can be performed [31, 32].

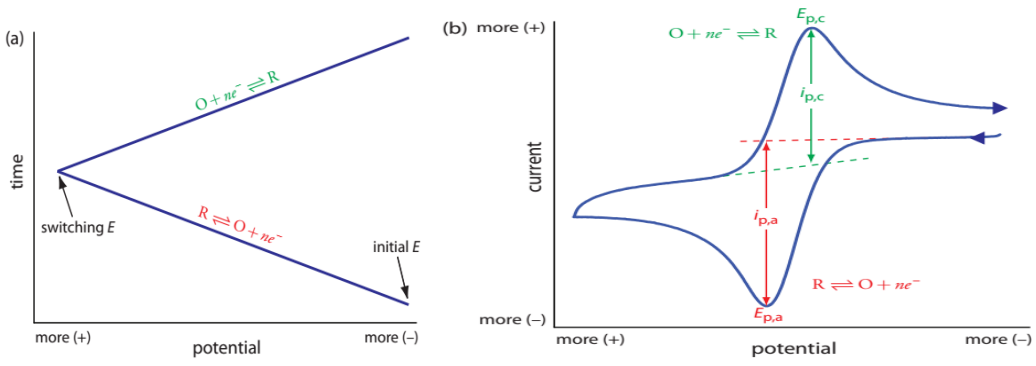


Figure 1. (a) One cycle of the triangular potential-excitation signal showing the initial potential and the switching potential. b) The resulting cyclic voltammogram showing the measurement of the peak currents and peak potentials.

The important parameter of a cyclic voltammogram are the magnitude of the anodic peak current (I_{pa}), Cathodic peak current (I_{pc}), anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}). A redox couple in which both species (reduced and oxidized) rapidly exchange electrons with the working electrode is termed as electrochemical reversible couple. Such couple can be identified from a cyclic voltammogram by measurement of the potential difference between the two peaks potential. The formal reduction potential E$^{o'}$ for reversible couple is centered between E_{pa} and E_{pc} [31, 33].

$$E^{o'} = \frac{E_{pa} + E_{pc}}{2} \qquad 1$$

The number (n) of electrons transferred in the electrode reaction for reversible couple can determined from separation between peak potentials.

$$dE = E_{pa} - E_{pc} = \frac{59}{n} mV \qquad 2$$

Where n is the number of electrons transferred and E_{pa} and E_{pc} is the anodic and cathodic peak potential, respectively, in volts. Thus for reversible redox reaction at 25 ^{o}C dE should be 59 mV or about 60 mV for one electrons.

The dependence of the anodic peak current density on the scan rate has been used for the estimation of the ‘‘apparent’’ diffusion coefficient, D_{app}, for the compounds under consideration. D_{app} values were calculated from the Randles Sevcik equation,and for the oxidized species [O]:

$$I_p = 0.4463(F^3/RT)^{1/2} n^{3/2} \upsilon^{1/2} D_0^{1/2} A C_0 \qquad 3$$

For T = 298 K (at which temperature the experiments were conducted), the equality holds true:

$$I_{pa} = (2.69 \times 10^5)\, n^{3/2} A C_0 * D_0^{1/2} \upsilon^{1/2} \qquad 4$$

Where the constant has the units: 2.687×10^5 C mol^{-1} V$^{-1/2}$. In these equations: Ip is the peak current density (A cm^{-2}), n is the number of electrons appearing in the half-reaction for the redox couple, υ is the rate at which the potential is swept (V s^{-1}), F is Faraday’s constant (96 485 C mol^{-1}), C_0 is the analyte concentration (1×10^{-6} mol cm^{-3}), A is the electrode area (0.0706 cm^2), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute T/K, and D is the electroactive

species diffusion coefficient ($cm^2\ s^{-1}$). Apparent surface area used in the calculations did not take into account the surface roughness [34].

For irreversible processes (those with sluggish electron exchange), the individual peaks are reduced in size and widely separated, i.e., the separation of peak potentials is greater than 59/n mV. The peak current is given by:-

$$I_p = (2.99x10^5)\ \alpha^{1/2} n^{3/2} A C D^{1/2} \upsilon^{1/2} \qquad 5$$

Where α is the transfer coefficient and n is the number of electrons involved in the charge transfer step, is still proportional to the bulk concentration, but the voltammogram peak obtained lower in height. For totally irreversible systems, the reverse scan may not reveal any peak.

2.3.2. Pulse Voltammetry

The basis of all pulse techniques is the difference in the rate of the decay of the charging and the faradaic currents following a potential step (or "pulse"). The charging current decays exponentially, whereas the faradaic current (for a diffusion-controlled current) decays as a function of 1/ (time)^{½}; that is, the rate of decay of the charging current is considerably faster than the decay of the faradaic current.

2.3.2.1. Normal Pulse Voltammetry

This technique uses a series of potential pulses of increasing amplitude. The current measurement is made near the end of each pulse, which allows time for the charging current to decay. It is usually carried out in an unstirred solution at either DME (called normal pulse polarography) or solid electrodes. The potential is pulsed from an initial potential E_i. The duration of the pulse, t, is usually 1 to 100 msec and the interval between pulses typically 0.1 to 5 sec. The resulting voltammogram displays the sampled current on the vertical axis and the potential to which the pulse is stepped on the horizontal axis [31].

2.3.2.2. Differential pulse voltammetry

This technique is comparable to normal pulse voltammetry in that the potential is also scanned with a series of pulses. However, it differs from NPV because each potential pulse is fixed, of small amplitude (10 to 100 mV), and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, the first point (i) just before the application of the pulse and the second (ii) at the end of the pulse (**Figure** 2). These sampling points are selected to allow

for the decay of the nonfaradaic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential. DPV is useful due to eliminations in the contribution of non-faradaic (capacitive) processes, which are effectively subtracted out. Additionally, DPV is useful for resolving the voltammetric signals due to twospecies with close half-wave potentials, producing easily quantifiable peak shaped responses [35].

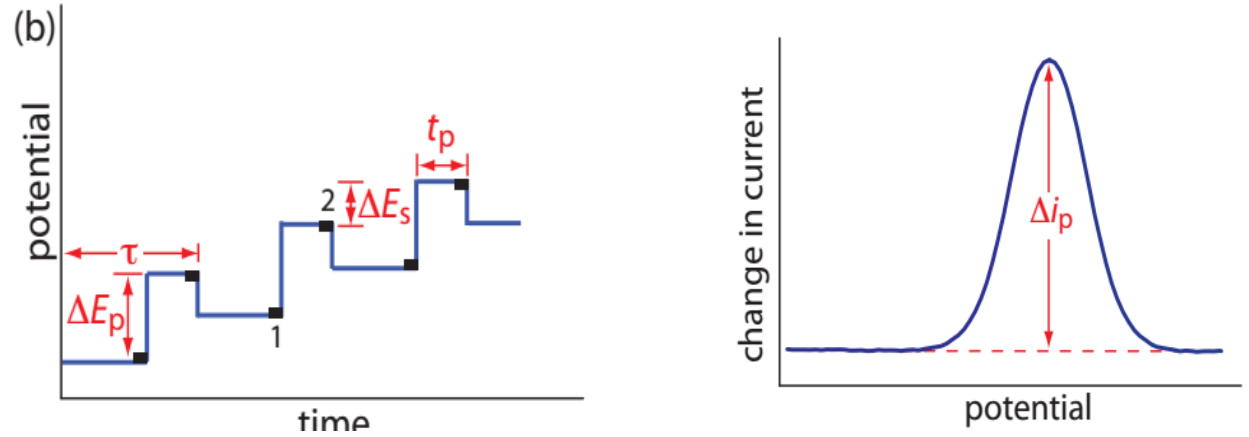


Figure 2. (a) Excitation wave form and (b) response obtained by differential pulse voltammetry. Where τ is the cycle time; ΔE_p is a fxed or variable pulse potential; ΔE_s is the fxed change in potential per cycle, and t_p is the pulse time.

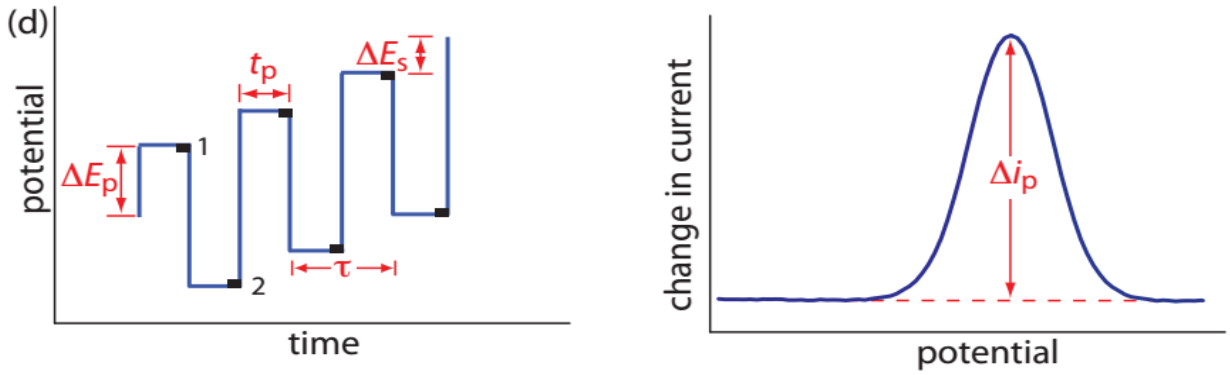
2.3.2.3. Square -Wave Voltammetry

The square wave voltammetric waveform consists of a square wave superimposed on a staircase. The currents at the end of the forward and reverse pulses are both registered as a function of staircase potential (**Figure** 3). The difference between them, the net current, is larger than either of its two component parts in the region of the peak which is centered on the half-wave potential. Capacitative contributions can be effectively discriminated against before they die away, since, over a small potential range between forward and reverse pulses, the capacitance is constant and is thus annulled by subtraction. In this way the pulses can be shorter than in DPV and the square wave frequency can be higher [36].

Detection limits of 10^{-8} M or lower are readily achievable under optimum conditions. The advantages over cyclic voltammetry are as follows: faster scan rates are possible (faster reactions

can be studied), higher sensitivity (lower concentrations can be used) and a higher dynamic range (a larger range of concentrations can be investigated). Usually in electrochemistry, solutions are vigorously degassed with, for example, nitrogen to remove oxygen which is electrochemically reduced and can interfere with the voltammetric measurement under investigation. A different way of greatly diminishing or eliminating the interference of oxygen, with no need for its removal, is by the use of the high frequencies employed in SWV. In fact, due to the irreversibility of oxygen reduction, the increase of its signal with frequency is small at high frequencies, and becomes negligible eventually, when compared with the response of the determinant [37].

Square wave voltammetry can be used to perform an experiment much faster than normal and differential pulse techniques, which typically run at scan rates of 1 to 10 $mVsec^{-1}$ and allowing much faster determinations. A typical experiment requiring three minutes by normal or differential pulse techniques can be performed in a matter of seconds by square wave voltammetry [38].



(a) **(b)**

Figure 3. (a) Excitation wave form and (b) current vs potential plots of square wave voltammetry. The symbols in the diagrams are as follows: τ is the cycle time; ΔE_p is a fxed or variable pulse potential; ΔE_s is the fixed change in potential per cycle, and t_p is the pulse time.

3. Statement of the Problem

Many researchers at different time have been developed various methods to simultaneously determine CAF and PA by modifying glassy carbon electrode using organic polymer, inorganic complexes and different single and composite nanomaterials. These methods are expensive, in some case less selective and time consuming. Therefore, in this study very simple and cheap activated of glassy carbon electrode was conducted to determine CAF and PA simultaneously by using cyclic and square wave voltammetric techniques.

4. Objective

4.1. General Objective

- The general objective of this study is to determine CAF and PA by using AGCE simultaneously

4.2. Specific Objective

The specific objectives of this study are to:

- Compare the electrochemical behaviour of PA and CAF at bare GCE and AGCE
- Identify type of reaction (adsorption or diffusion) and optimal condition
- Mention the lowest detection and quantification limit of activated electrode and compared with result obtained at another electrodes
- Evaluate the feasibility of the method in real samples
- Study the sffects of interference on the determination ofCAF and PA

5. Significance of the Study

Firstly, this study is used to enhance application of AGCE in real sample analysis (pharmaceutical sample). Secondly, to give some information on AGCE and its effectiveness for simultaneous determination of CAF and PA for researcher.

6. Experimental Section

6.1. Instrumentation

The voltammetric experiments were done by using CV-CHI760E voltammetric analyzer [Bioanalytical Systems (BAS), USA] connected to a desktop with conventional three-electrode configuration. Electrochemically activated glassy carbon electrodes with a diameter of 3.0 mm was used as the working electrode; a platinum electrode served as the counter electrode with saturated Ag/AgCl electrode as the reference electrode. For preparation of the buffer solutions the pH was measured by a Jenway model 3510 pH meter and an electronic balance (Denver Instrumen. Glass road, mortar, pestle and flask are also used [39].

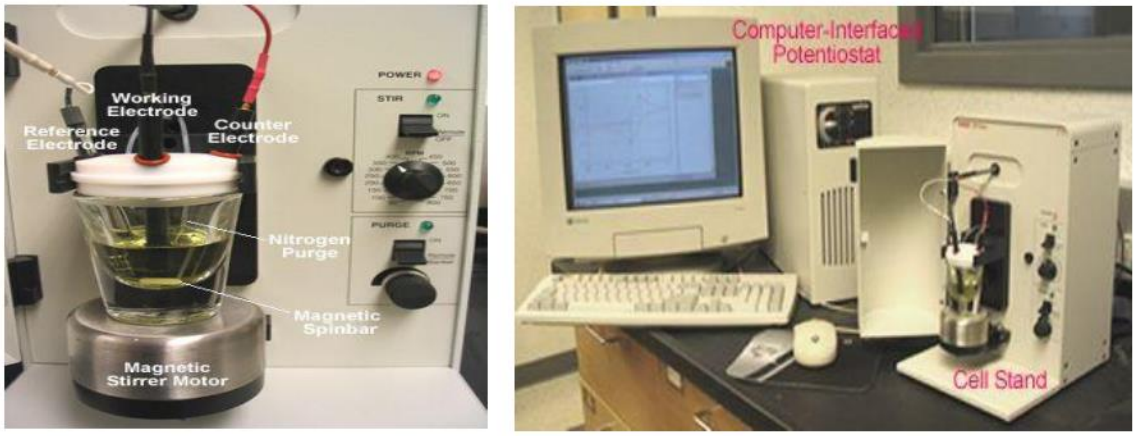


Figure 4. Potentiostat with computer interface

6.2. Analytical Procedure

6.2.1. Chemicals and Reagents

CAF (Godrej industries, India), PA (APF, Ethiopia), anhydrous di-potassium hydrogen orthophosphate (BDH, England), potassium dihydrogen phosphate (Sigma-Aldrich Switzerland), hydrochloric acid (Riedel-deHaen, Germany), sodium hydroxide(BDH, England) , and uric acid (Godrej industries, India) were used as received without any further purification. The stock solution of PA and CAF was prepared and stored in a refrigerator until used. An aqueous solution was prepared daily of the working days by simple dilution of the stock solution with phosphate buffer solution of pH 7. Phosphate buffer solutions (0.1 mM KH_2PO_4 and 0.1 mM K_2HPO_4) was

prepared by using distilled water. The pH of the phosphate buffer solution was adjusted by adding drop by drop of concentrated hydrochloric acid and sodium hydroxide to 0.1 mM PBS.

6.2.2 Preparation of the Activated Glassy Carbon Electrode

Before activation, the surface of GCE (3.0 mm diameter) was polished to a mirror with 0.5 μm alumina slurry (powder) on polishing cloth and then thoroughly rinsed with distilled water. The GCE was activated by applying a potential of 1750 mV for 200 s (optimum condition for electrochemical anodization) in time based technique in PBS. Cyclic voltammogram of the activated electrode was run between 0 to 700 mV until a stable voltammogram obtained [17, 26, 39].

6.2.3. Preparation of Stock and Standard Solutions

A stock solution of PA and CAF (1 mM) was prepared by dissolving 0.0750 g PA and 0.0971 g CAF in 500 mL of PBS . A series of standard solutions was prepared from the stock solution by dilution with the PBS of pH 7 [40].

6.2.4. Preparations of Calibration curve

To prepare a calibration curve of CAF and PA, different concentration of PA (10 μM - 180.0 μM) at 0.5 mM CAF was prepared by PBS at pH 7. In same way different concentration of CAF (100 μM - 950 μM) at 10 μM PA was prepared by PBS. SWV at square wave amplitude 25 mV, frequency (15 Hz) and with potential window -200 to 1600 mV of each prepared solution was measured. Their calibrations curves were drawn using origin 8.

6.2.5. Sample Preparation from Tablets

Commercially available tablets known as SNIP pain (which contains 500 mg PA and 65 mg PA per tablet) was purchased from pharmaceutical drug shop. Five tablet formulations were accurately weighed and ground using mortar and pestle. An adequate amount of this powder (0.172 g), was weighed and transferred into a 500 mL flask and filled to the mark with 0.1 mM pH 7 PBS. Concentration of each sample (CAF and PA) present in the solution were calculated (1.7 mM PA and 0.17 mM CAF). In the prepared solution the concentration of PA was out of range of calibration curve of its standard solution, therefore the working solution of PA (51 μM in 50 mL volumetric flask) and (790 μM CAF) were prepared by using dilution formula. Then the prepared solution was filled up to the mark by using PBS.

6.2.6. Method of Analysis

Cyclic voltammetry in the potential window 1800 to -400 mV was used for the investigation of the electrochemical behavior of standard CAF and PA at bare GCE and AGCE electrode. The effects of scan rate in the range of 20 to 300 mV/s and pH in the range 2 - 10 on the peak potential and peak current of CAF and PA were also studied using cyclic voltammetry. For the quantitative determination of CAF and PA at AGCE as a working electrode, a square wave voltammetry in the range 1500 to -200 mV was employed. Linear calibration curve for the dependence of square wave peak current on the concentration of standard CAF and PA were studied. Recovery results of spiked standard CAF and PA in tablet solutions, interference study results, method detection limit, linear range of the results obtained were used to validate the applicability of the developed method for the simultaneous determination of CAF and PA in pharmaceutical formulations.

7. Results and Discussion

7.1. Cyclic voltammetric Behaviour of CAF and PA at Bare GCE and AGCE

Cyclic voltammetric behaviour of 0.3 mM PA at bare GCE was studied in 0.1 mM PBS, pH 7.0 at a scan rate of 100 mV/s. PA exhibit a irreversible behaviour at bare GCE with anodic peak potential at 0.37 mV. However at AGCE, a well resolved reversible anodic and cathodic peaks appear at 0.365 mV and 0.345 mV, respectively with ΔEp of 0.02 mV (**Figure** 5), along with enhanced peak current response this is a clear evidence of the electrocatalytic effect of the AGCE towards the oxidation of PA due to the fast electron transfer kinetics at AGCE.

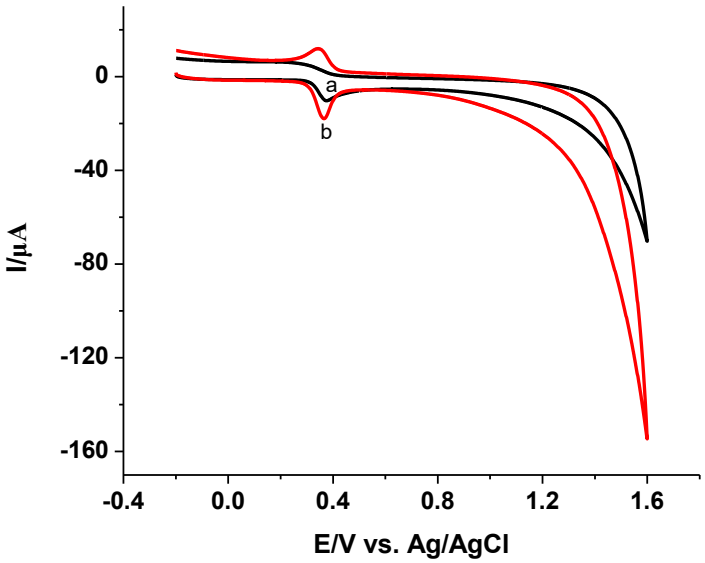


Figure 5. Cyclic voltammogram of 0.3 mM PA at a) bare GCE and b) AGCE in 0.1 mM PBS of pH 7 at scan rate of 100 mV/s

Activation of an electrode can be shown a good electrocatalytic effect on the oxidation of PA in which anodic peak potential was shifted to negative value and the magnitude of oxidation peak current was increased.

Even though CAF oxidation peak potential was shifted to negative value, as shown on **Figure** 6 and 7 unresolved and weak oxidation peak current of was observed at surface of AGCE, indicate

that the weak interaction between CAF and surface of AGCE, this may due to blocking of the electrode surface by the oxidation product [23].

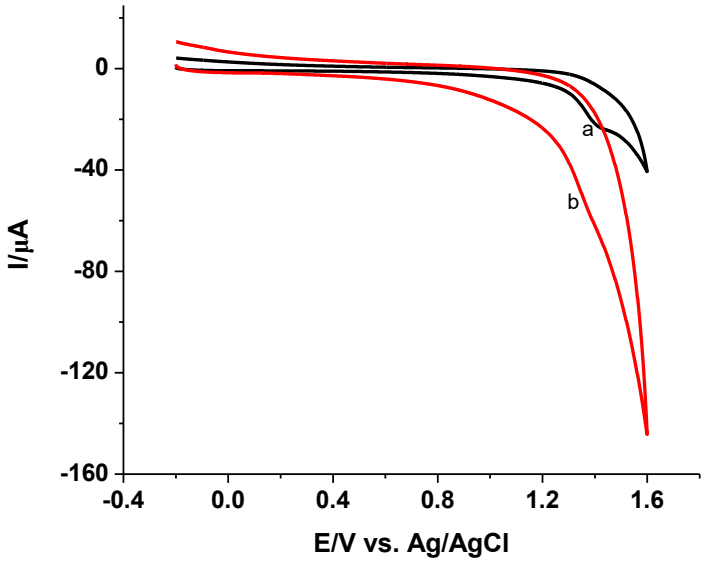


Figure 6. Cyclic voltammogram of 0.3 mM CAF at a) bare GCE and b) AGCE in 0.1mM PBS of pH 7 at scan rate of 100 mV/s

The mixture of PA and CAF were also studied at both bare and AGCE. As shown in **Figure** 7 the well defined reversible oxidation peak current were observed at AGCE for PA, with slight shift of oxidation peak potential toward negative value. Similarly, the oxidation peak potential of CAF was shift to negative value which show the good electrocatalytic effect of AGCE. Therefore, AGCE electrode was used for simultaneous dermination of both drug in the same mixture in subsequent study.

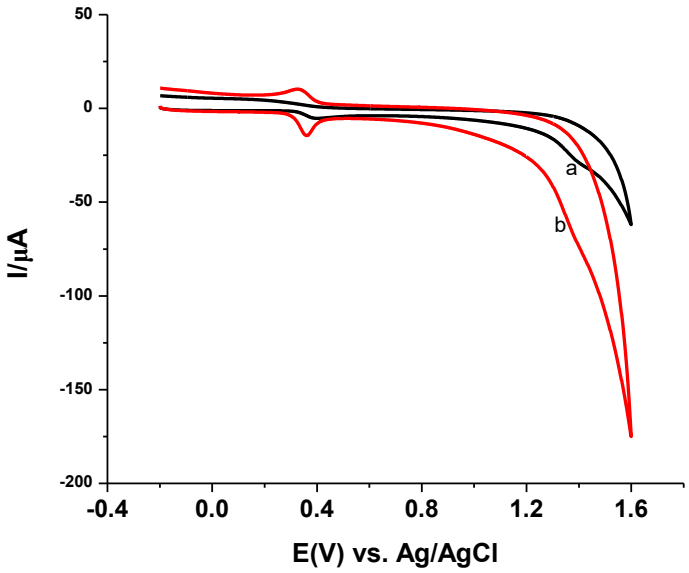


Figure 7. Cyclic voltammogram of 0.3 mM mixture of CAF and PA at a) bare and b) activated GCE 0.1 mM PBS of PH 7 at scan rate of 100 mV/s

7.1.1. Effect of pH of Buffer Solution on Electrochemical Behaviour of CAF & PA

The effect of PBS pH on the electrochemical response of the sensor toward the simultaneous determination of CAF and PA was studied by cyclic voltammogram within the pH range of 4 -10. The results obtained are presented in **Figure** (8). As it can be seen from **Figure** (9A), the PA oxidation peak current is increased sharply as the pH increased to 7 and then decreased up to pH 10, but for CAF oxidation peak current decreased up to pH 7 then start to increase up to pH 8 then remained constant to pH 10. As shown on the **Figure** 9A the optimum pH for PA is 7 (the pH at which maximum peak current of PA was observed), but it is not the optimum pH for CAF. The maximum peak current was observed for CAF at pH 4. Therefore, it is impossible to use the oxidation peak current as evidence to judge the optimum pH. Compared to other pH value, CAF has well defined oxidation peak and in good separation with PA at pH 7. Therefore, pH 7 was chosen as optimum pH for simultaneous determination of CAF and PA for subsequent work.

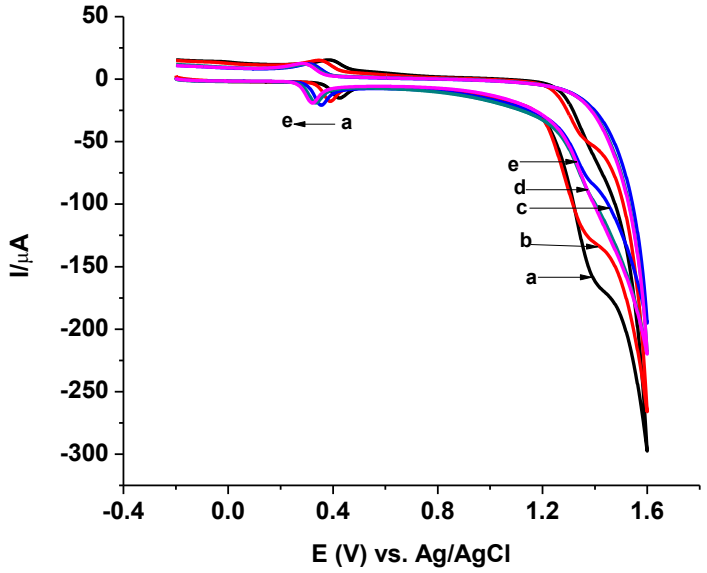


Figure 8. Cyclic voltammogram of 0.3 CAF and PA at activated GCE in 0.1 mM PBS of different pH (a) 4, (b) 6, (c) 7, (d) 8, and (e) 10 at 100 mV/s

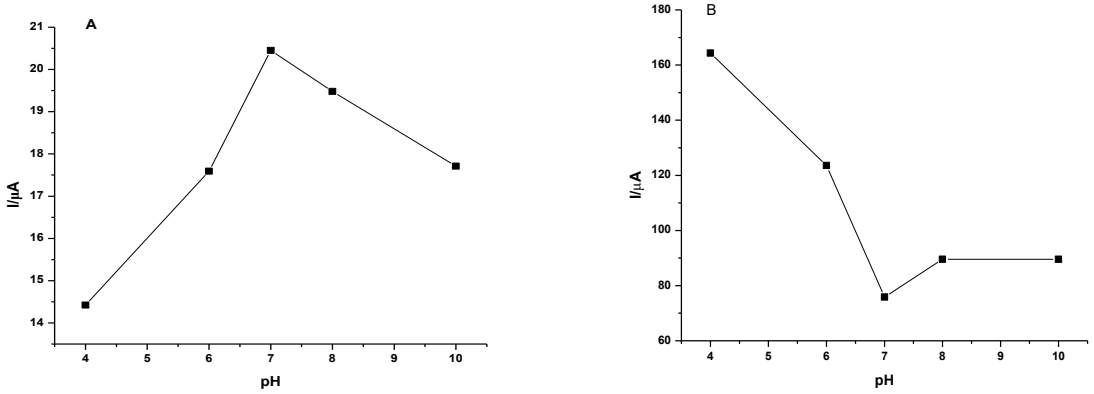


Figure 9. Plot of effect of pH on oxidation peak current of A) PA and B) CAF at AGCE in 0.1 mM PBS, pH range of 4.0-10.0 at a scan rate of 100 mV/s.

The pH of the PBS has a significant influence on the oxidation peak potential of the catalytic oxidation of PA and CAF, i.e. the anodic peak potentials (E_{pa}) of PA was shifted negatively with increase of the solution pH, this indicates that the electrocatalytic oxidation at the AGCE is a pH-dependent reaction and that protons have taken part in their electrode reaction processes. Similarly, the peak potential of CAF shift to negative value as the pH varies from 4 to 7 this indicate good electrocatalytic effect of AGCE toward CAF with involvement of proton , then again it start to raise to positive value up to pH 10, this indicate the absence of proton in their electrode reaction when PBS was in more basic condition. AGCE is more effective toward catalysis of CAF almost in neutral condition, therefore this is one evidence why neutral PBS (pH 7) was selected as optimum pH.

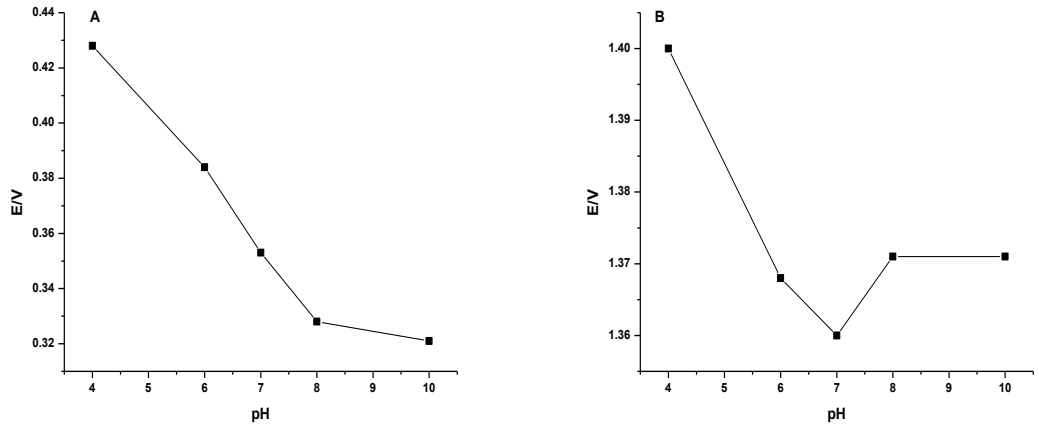


Figure 10. Plot of effect of pH on oxidation peak potential of A) PA and B) CAF at AGCE in 0.1 mM PBS, pH range of 4.0-10.0 at a scan rate of 100 mV/s.

The peak potential for PA and CAF oxidation varies linearly over the pH range from 4 – 8 and 4 – 7 respectively.

CAF: Epa = -0.01457pH + 1.457 PA: E_{pa} = -0.02517pH + 0.53057 (R^2 = 0.9928)

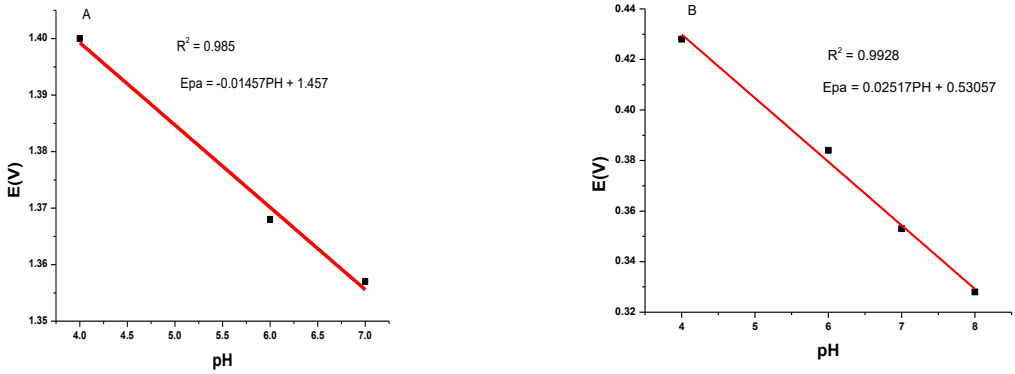
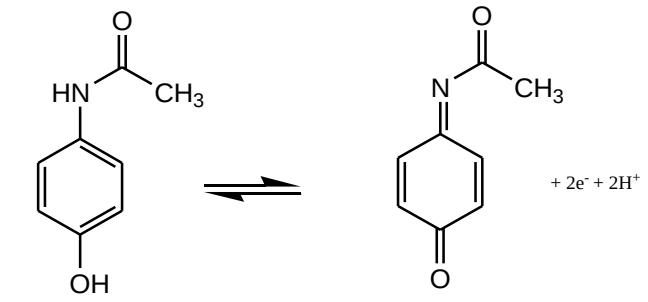


Figure 11. plot of Linear variation range of oxidation peak potential with pH of 0.1 mM PBS A) CAF B) PA

The slope of these graphs should be equal to -59 p/n where p is the number of protons involved in the electrode reaction, n is the number of electrons transferred. In this study, the p/n values for PA and CAF were calculated as 0.42 and 0.25.

As paracetamol oxidation is known to involve two protons and two electrons, the slope would be expected to be 59 mV pH^{-1}. The 25.17 mV pH^{-1} slope obtained in the present studies indicates that the electrode process is more complex and is caused by the fact that small quantity of PA is oxidised to N-hydroxy acetaminophen [23]. Therefore this study was shown that electrode reaction process was close to one proton and two electron process.

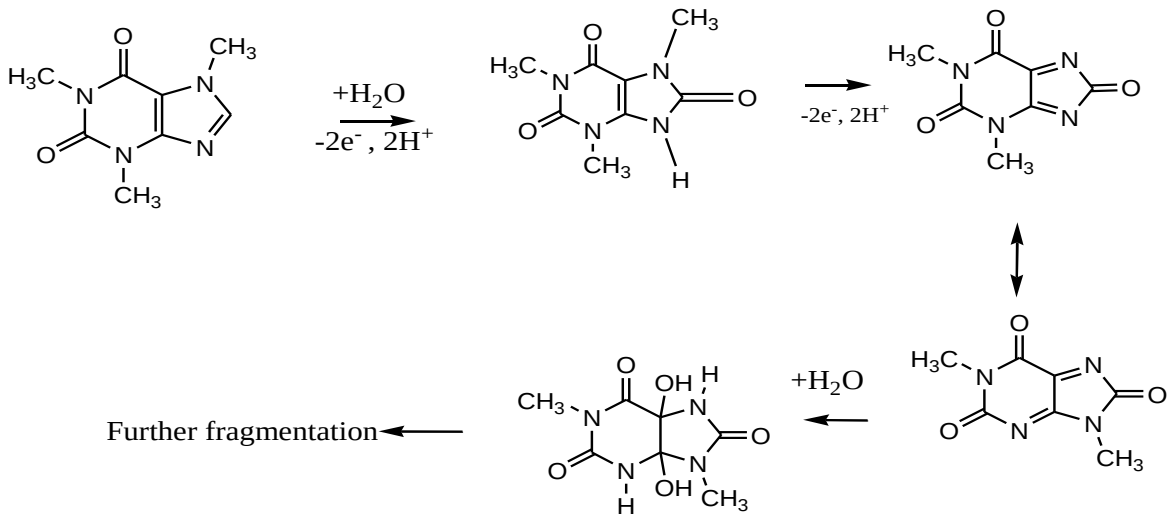


paracetamol N-Acetyl-p-quinoneimine

Scheme 7. Electrochemical oxidation of PA

The electrochemical oxidation of CAF is an overall $4e^-$, $4H^+$ process. A $2e^-$, $2H^+$ oxidation of the C-8-N-9 bonds was performed to give the substituted uric acid. The electrochemical oxidation of

substituted uric acid proceeds rapidly in a $2e^-$, $2H^+$ process to lead to an unstable diimine species which is then attacked by water molecules in a step-wise fashion to be converted into an imine-alcohol and then substituted uric acid-4, 5 diol. The uric acid-4, 5 diol compound produced is unstable and decomposes to various products depending on the solution pH [24, 25].



Scheme 8. Electrochemical oxidation of CAF

Scheme 8 show the probable electrochemical oxidation of CAF. In this study CAF involved unequal number of protons and electrons in the oxidation process. CAF slope -14.57 mV per pH unit indicates that the electrode process is more complex in the studied pH window and the ratio of number of protons and transferred electrons is not equal to 1 and involve less protons than electrons i, e., 4 electrons and one proton. Although the reasons for this behaviour is unclear, this could be explained as the interference due to uncertainties introduced by the close proximity of voltammetric peak to the background discharge probably due to oxygen evolution or the product of oxidation undergoing deprotonation [23].

7.1.2. Effect of Scan Rate on Electrochemical Behaviour of CAF and PA

The study of the influence of scan rate on the peak current and peak potential with electrochemically activated glassy carbon electrode was also carried out for CAF and PA within the range of 20 to 300 mV s^{-1}. In order to investigate whether the oxidation process of CAF and PA at AGCE is predominantly diffusion controlled or adsorption controlled process, the correlation coefficients for the linear plots of the oxidation peak current versus the scan rate and square root of scan rate were compared.

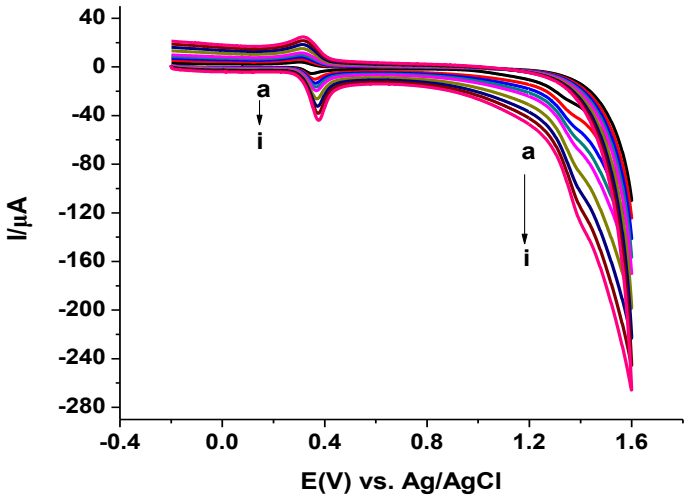


Figure 12. Cyclic voltammogram of effect of scan rate (a) 20, b) 40 c) 60, d) 80, e) 100, f) 150, g) 200, h) 250, i) 300) on oxidation current and oxidation potential of 0.3 mM CAF and PA in 0.1 mM PBS of pH 7 at AGCE

It was found that the peak current of CAF increased linearly with the square root of scan rate, with correlation coefficient of (R^2 = 0.996) and scan rate with correlation coefficient (R^2 = 0.992 **Figure** not shown). No voltammetric signal was observed, when the working electrode was switched to a medium containing only supporting electrolyte after being used in voltammetric measurements of caffeine solution, these indicates that the reaction process of CAF with AGCE was diffusion controlled. This result was in agreement with the data previously reported [41].

The linear variation of the peak current with the square root of the scan rate is described by the equation:

$I_p = -8.786v^{1/2} + 21.51$ (μA), (R^2= 0.996).

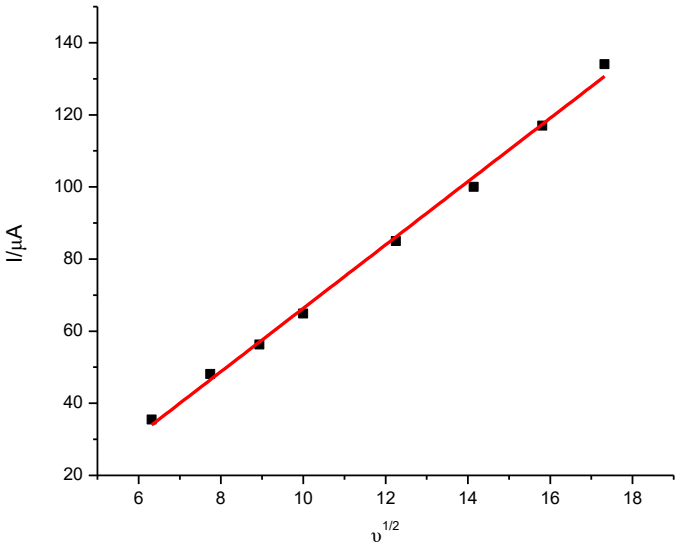


Figure 13. plot of effect of square root of scan rate (6.32, 7.74, 8.94, 10, 12.25, 14.14, 15.81, 17.32) on peak current of 0.3 CAF in 0.1mM PBS of PH 7 at AGCE

According to simplified Randles- Seviks equation (I_p = constant $\times \upsilon^{1/2}$), a linear plot of log I_p versus logυ was obtaining with the following equation: log I_p = -5.472 + 0.64 logυ

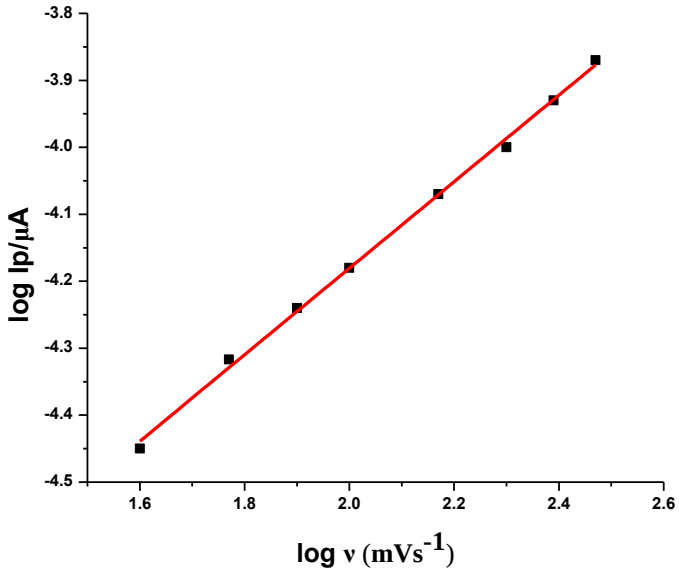


Figure 14. Plot of log I_p vs log υ of CAF

The slope value of 0.64 was close to the theoretically expected value of 0.5 for a purely diffusion controlled process, which, further confirms that the electrocatalytic oxidation process of CAF at AGCE is controlled by diffusion which received a good support from the literature [41].

The linear variation of the peak potential with the logarithm of the scan rate is also described described by the equation: E_p = 0.0593 lnv + 1.2594 (V), (R^2 = 0.98314) (**Figure** 15). Caffeine oxidation peak potential was observed to shift positively with increase in scan rate, which confirms irreversibility of the oxidation processes. The result indicates that the oxidation of CAF at the electrochemically AGCE is diffusion-controlled irreversible oxidation process.

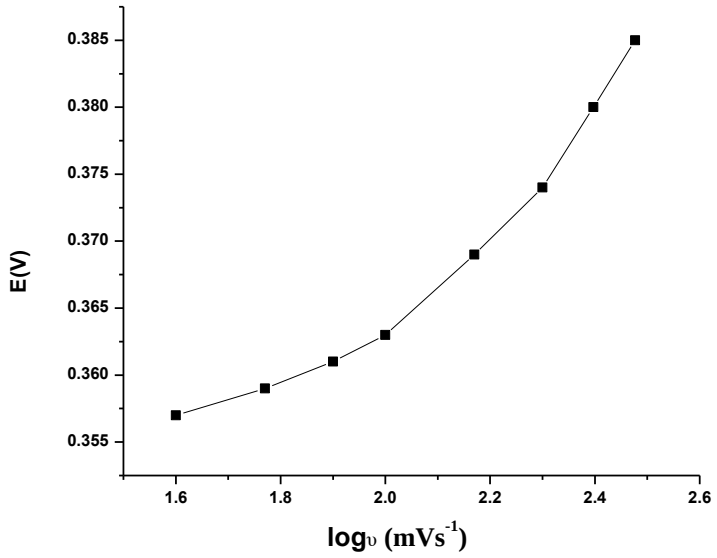


Figure 15. Plot of Peak potential vs log of scan rate (1.6, 1.78, 1.9, 2, 2.18, 2.3, 2.39, and 2.48) of CAF in 0.1mM PBS of pH 7 at AGCE

The logarithim of peak current vs logarithim of scan rate was also plotted for PA in order to decide wether the electrode reaction process is diffusion or adsorption controlled. As shown in the **Figure** 16 the slope (0.748 and 0.7004) of logIpa and Ipc versus log scan rate was between 0.5 and 1. Even if the obtained slope for both log of oxidation and reduction peak current versus log of scan rate was more close to 0.5 (theoretical value for diffusion controlled process), to be sure still additional information is needed.

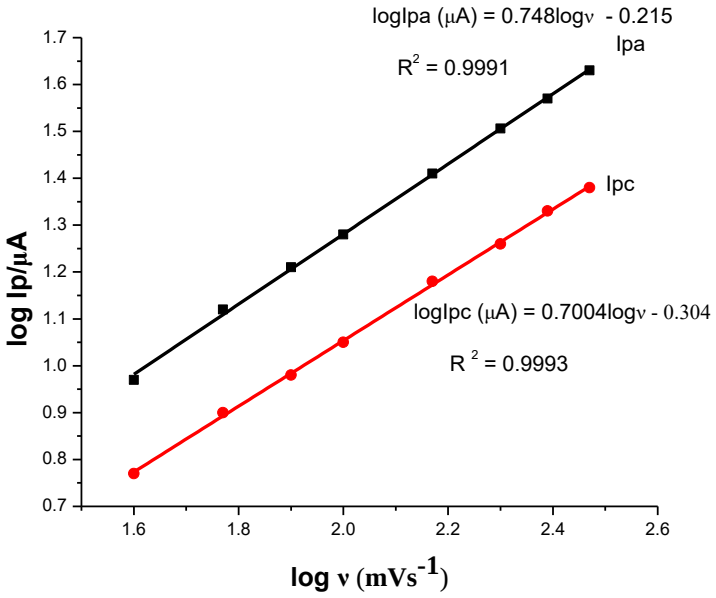


Figure 16. Plot of log I_p vs log υ of PA

It is clear that both the oxidation and reduction peak currents of PA increased with increasing the square root of scan rate and scan rate. A linear relationship between the anodic and cathodic peak currents and scan rate of PA was found as follows: I_{pa} (μA) = -0.12825ν (mVs^{-1}) - 5.8127 (R^2= 0.99412) and I_{pc}(μA) = 0.0700υ + 3.991 (R^2 = 0.99173). A linear relationship between anodic and cathodic peak currents and square root of scan rate was found as follows: Ipa(μA) = -3.065υ$^{1/2}$ (mVs^{-1}) + 10.72 (R^2 = 0.9967) and Ipc (μA) = 1.6759υ$^{1/2}$ (mVs^{-1}) – 5.064 (R^2 = 0.9979).

The correlation coefficient of curve between peak current versus square root of scan rate(fig. 17A) and the slope of logIpa &Ipc versus logυ (fig.16) suggesting that redox reactions of CAF and PA compounds at AGCE, are diffusion-controlled process.

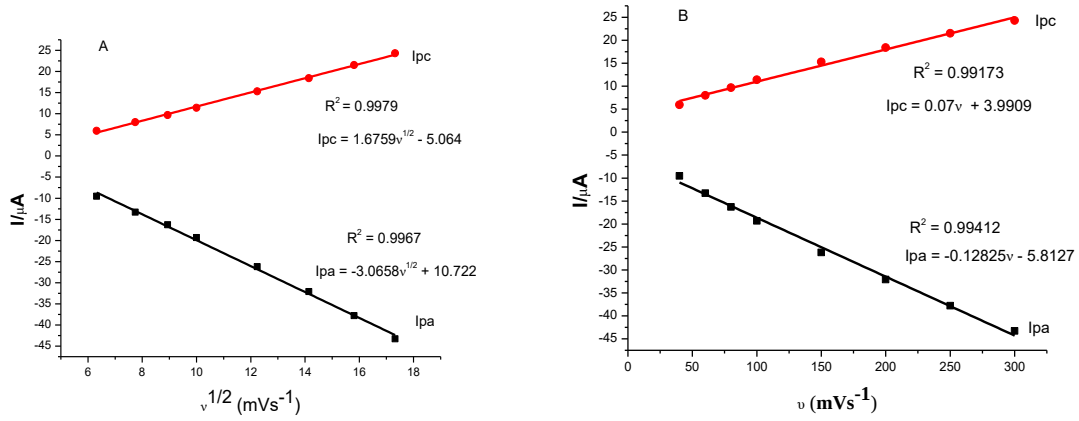


Figure 17. Plots of the oxidation and reduction peak current of the PA and the A) square root of scan rate B) scan rate

7.2. Simultaneous determination of CAF and PA by SWV

Caffeine is present along with PA in most of the analgesic formulations. Therefore, simultaneous determination of them is important in pharmaceutical point of view. Square wave voltammetric (with SW Amplitude of 25 mV, Frequency 15 Hz, scan increment 4 mV, quiet time of 2 s) experiments were carried out to further investigate the electrochemical behaviour of CAF and PA were present in same solution. The SW voltammogram presented oxidation peaks at 0.32 V for PA and 1.32 V for CAF (*vs* Ag/AgCl). The peak separation of about 1.0 V clearly allows the simultaneous determination of these compounds.

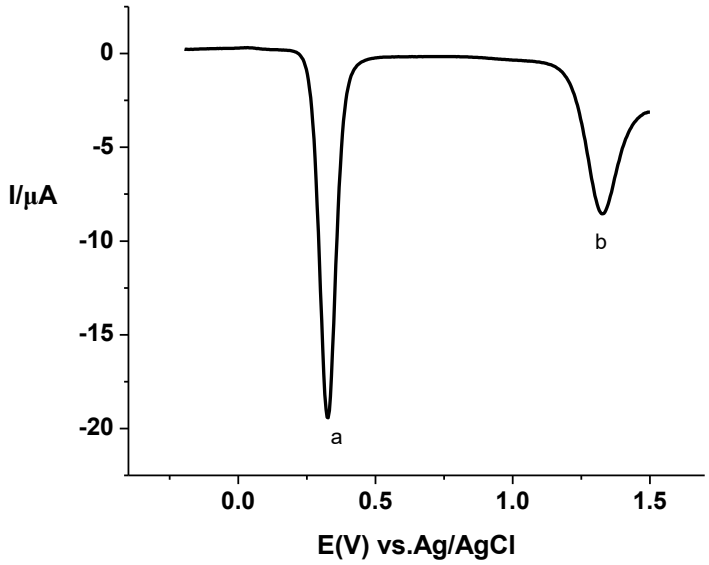


Figure 18. SW voltammogram of a) 0.3 mM PA and b) 0.3 mM CAF in 0.1mM PBS at pH 7 using AGCE with SW Amplitude of 25 mV, Frequency15 Hz, scan increment 4 mV and quiet time of 2 s.

To further investigate the electrochemical solution when both substances are present in solution, SW voltammogram were obtained in the presence of PA or CAF in the 0.1 mM PBS of pH 7, as presented in **Figure** (19). Determination of CAF was performed with concentration range between 100 and 950 µM, in solutions containing 10 µM PA and its oxidation peak current increase linearly as its concentration increase from 100 to 950 µM with calibration equation of $Ip(\mu A) = 37.21C_{caf} + 1.577$ ($R^2 = 0.991$) (**Figure** 19B). Similarly, determination of PA in concentrations between 10 and 180 µM was accomplished in solutions containing CAF at fixed concentration of 0.5 mM **Figure** (19A). An examination of **Figure** 19A, allows concluding that the oxidation peak current for PA increases regularly as its concentration is increased from 10 to 180 µM, in the presence of a fixed CAF concentration (0.5 mM) with calibration equation of $Ip\ (\mu A) = 0.184C_{pa} + 2.07$ ($R^2 = 0.990$). The CAF peak current remains fairly constant, with Ip(CAF) = 17.36 ± 0.71 µA (n = 8). Similarly, as shown in **Figure** 19B, the peak oxidation current for CAF increases regularly as its concentration is increased in the presence of a fixed concentration of PA, whose oxidation peak current remains almost constant, with Ip(PA) = 2.06µA ± 0.3 (n = 8). Therefore, the enhancement

of concentration of one sample in the fixed concentration of others was not show significant change on the response of sample with fixed concentration.

The limits of detection (LOD) and limts of quantification (LOQ) were calculated as being 3(s/m), and 10 (s/m) respectively, where s is the standard deviation of 9 measurements performed for the supporting electrolyte alone and m is the respective analytical sensitivity (slope of analytical curve). The obtained LODs were: 2.55 µM for PA and 0.36 µM for CAF, and LOQ were: 8.49 µM for PA, and 1.21 µM for CAF.

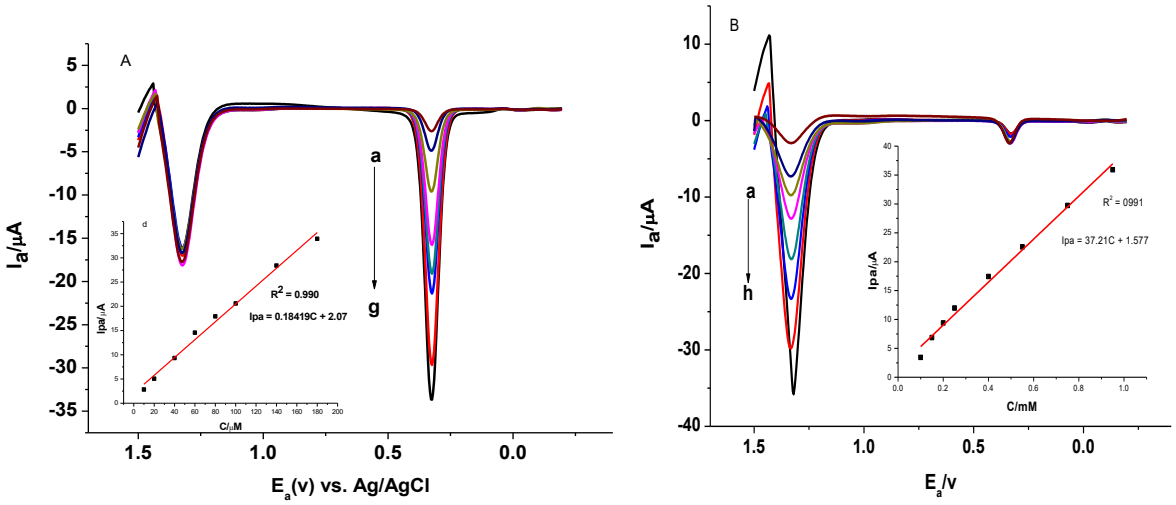


Figure 19. A) Square wave voltammogram of variable concentration of A) (a) 10, b) 20, c) 40, d) 80, e) 100, f)140 and g) 180 µM) PA at constant concentration (0.50 mM) CAF , B) square wave voltammogram of different concentration of (a)100, b) 150, c) 200, d) 250, e) 400, f) 550, g) 750 and h) 950 µM) CAF at constant concentration of 10 µM PA, in 0.1 mM PBS of pH 7 at AGCE with SW Amplitude of 25 mV, Frequency15 Hz, scan increment 4mV and quiet time of 2 s.

It is possible to observe that any change in the CAF signal occurs when PA concentration increases (**Figure** 19A). However the opposite situation is seen when CAF concentration is increased once the base line of PA is affected (**Figure** 19B). This means, that it is necessary to take care with the base line during the quantitative simultaneous analysis of these analytes. Such change can be a result of some interaction of CAF and PA (its oxidation products) with the working electrode surface.

7.3. Application of voltammetric methods in real samples

Commercial pharmaceutical formulation (tablet) containing different concentration of CAF and PA were analyzed to evaluate the validity of the proposed methods (SWV on electrochemically AGCE).

The square wave voltammograms for tablet sample solution containing 790 μM CAF and 51 μM PA was spiked with 55 μM CAF and 30 μM PA, were recorded. As can be observed from Table 1, recovery results 94.54% for CAF and 96.66% for PA confirmed the potential applicability of the developed method for CAF and PA analyses in real samples.

Table 1. Recovery of CAF and PA in drug sample

Matrix (tablet)	Added (μM)		Found (μM)		Recovery (%)	
	CAF	PA	CAF	PA	CAF	PA
SNIP pain	_	_	788	50	_	_
	55	30	840	79	94.54	96.66

7.4. Comparison of Proposed Technique with others

Table 2. Linear range and limits of detection for caffeine and paracetamol with other electrochemical methods.

Electrode	Method	Linear range of CAF/μM	Linear range of PA/μM	LOD of CAF/μM	LOD of PA/μM	Ref.
4α-Cu^{II}TAPc SAM modified GCE	DPV	5 – 1400		0.0304		[6]
Boron-doped diamond electrode	DPV	500 - 83000	500 - 83000	0.035	0.49	[15]
CuO–Gr/CPE	DPV	0.025 -5.30	0.025-5.30	0.01	0.008	[16]
DLC:VAMWCNT	SWV	9.97-91.7	0.997-36.7	0.367	0.334	[17]
Poly(Nile blue) modified glassy carbon electrode	DPV	0.8 - 20	0.2-162	0.1	0.08	[22]
Graphite and polyurethane screen-printed composite electrode	DPV	1 -200	1- 200	1.6	0.84	[39]
Poly(AHNSA)/GCE	SWV	10 - 125	10 – 125	0.79	0.45	[42]
Poly(taurine)/TiO_2-Gr/GCE	DPV	0.05- 100	0.05- 100	0.5	0.034	[43]
AGCE	SWV	100 -950	10 - 180	0.36	2.55	This work

Table 2 was evident that the proposed electrode has a wider linear range , LOD and more sensetive than some previously reported work at modified electrodes. Therefore these method is competitive with other reported technique for determination of PA and CAF in the same solution.

7.5. Interference Studies

There are various possible interferents which inhibit the determination of caffeine and paracetamol. One of the well-known interferent which highly affect the peak current and peak potential of PA and CAF is Uric acid. In this study PA and CAF examined in the presence of equimolar uric acid. As observed from the **Figure** 20, uric acid was highly affect the peak current of both PA and CAF (minimize from -29.94 to -27.01μA for PA and -18.36 to -14.12 μA for CAF), and with slight shift of peak potential of CAF toward negative side (1.36 to 1.34). In addition the relative error (change in oxidation peak current) caused by uric acid was listed in table 3. The signal change caused by addition of uric acid was greater than 5% which indicate the positive interferent effect.

Table 3. Interference study of CAF and PA with uric acid

Interferent	Concentration in μM of the interferent added to 300/μM CAF and PA	Recorded signal (Ip/μA) for CAF	Recorded signal (Ip/μA) for PA	Signal change (%) for CAF	Signal change (%) for PA
Uric Acid	0	18.36	29.94	_	_
	300	14.12	27.01	23.09	9.78

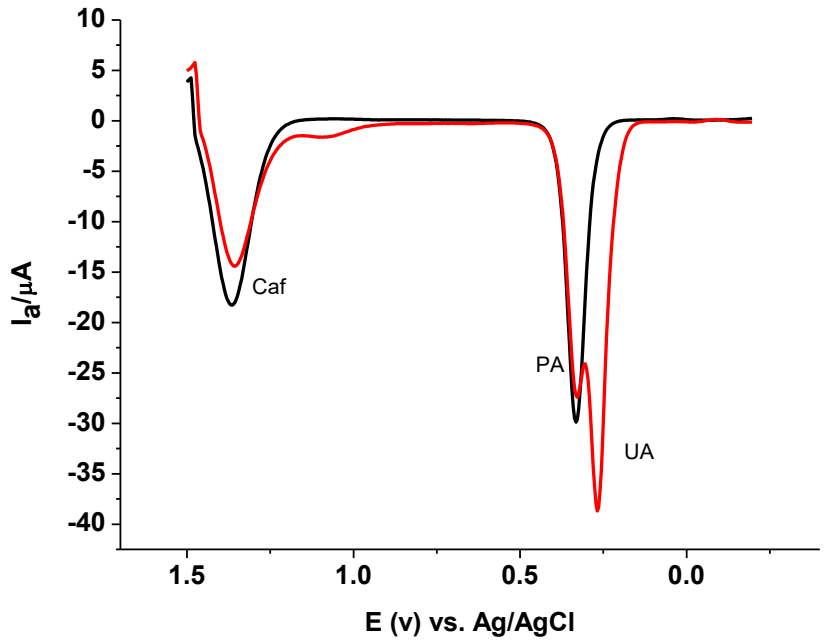


Figure 20. Effect of 0.3 mM Uric acid on the oxidation peak current and peak potential of 0.3 CAF and PA mixture in 0.1mM PBS of PH 7 at AGCE with SW amplitude of 25 mV, frequency 15 Hz, can incement 4 mV and quiet time of 2s

8. Conclusion

In the present study electrochemical behaviour of AGCE was compared with bare GCE electrode for individual and simultaneous determination of CAF and PA. As discussed, the electrochemical behaviour of both studied analyte was improved at AGCE as compared to bare GCE. This due to enhanced electrocatalytic effect of AGCE toward CAF and PA. The oxidation peak current of PA and CAF were increased proportionally with square root scan rate this indicate that reaction process at AGCE was diffusion controlled. SWV was used for determination of CAF and PA as the concentration of one was varied and the other kept constant. The result was shown that it was possible to determine CAF and PA simultaneously by using AGCE with LOD of 0.36 μM and 2.55 μM respectively. The recovery obtained (94.54% for CAF and 96.66% for PA) shown that the AGCE has the capability of determining CAF and PA in mixture and its significant application in real sample analysis. Uric acid has significant effect on the oxidation peak current and too small extent oxidation peak potential of CAF and PA.

9. Recommendation

Different pharmaceutical and biological samples contain CAF and PA in different dose. Therefore, the accurate determination of these drugs concentration is very important for quality control purpose. In this study the AGCE was used for the determination of these drugs simultaneously. But this method (AGCE) was unable to determine CAF and PA in the presence of Uric acid which can affect the oxidation peak current and peak potential of both drugs. Therefore this is good chance for the next researcher who will have interest, to design new sensor which have the capacity of determining both analyte in same mixture without interfering effect of uric acid.

10. References

1. Keeley, G.P., McEvoy, N., Nolan, H., Kumar, S., Rezvani, E., Holzinger, M., Cosnier, S. and Duesberg, G.S., 2012. Simultaneous electrochemical determination of dopamine and paracetamol based on thin pyrolytic carbon films. *Analytical Methods*, 4(7*)*: 2048.

2. Babaei, A., Dehdashti, A. and Afrasiabi, M., 2011. Development of a Method for a Sensitive Simultaneous Determination of Dopamine and Paracetamol in Biological Samples and Pharmaceutical Preparations. *International Journal of Electrochemistry*, 2011: 1.

3. Alothman, Z.A., Bukhari, N., Wabaidur, S.M. and Haider, S., 2010. Simultaneous electrochemical determination of dopamine and acetaminophen using multiwall carbon nanotubes modified glassy carbon electrode. *Sensors and Actuators B: Chemical*, 146(1): 314.

4. Tadayon, F., Naghinejad, R. and Daneshinejad, H., 2016. A Sensitive and Selective Electrochemical Method for the Simultaneous Determination of Dopamine and Paracetamol based on a Multiwalled Carbon Nanotubes/Poly (l-lysine)-modified Glassy Carbon Electrode. *Chemistry Letters*, 45(8): 1006.

5. Amare, M. and Aklog, S., 2017. Electrochemical Determination of Caffeine Content in Ethiopian Coffee Samples Using Lignin Modified Glassy Carbon Electrode. *Journal of Analytical Methods in Chemistry*, 2017: 2

6. Jeevagan, A.J. and John, S.A., 2012. Electrochemical determination of caffeine in the presence of paracetamol using a self-assembled monolayer of non-peripheral amine substituted copper (II) phthalocyanine. *Electrochimica Acta*, 77: 137.

7. Newton, R., Broughton, L.J., Lind, M.J., Morrison, P.J., Rogers, H.J. and Bradbrook, I.D., 1981. Plasma and salivary pharmacokinetics of caffeine in man. *European journal of clinical pharmacology*, 21(1): 46.

8. Meyer, F.P., Canzler, E., Giers, H. and Walther, H., 1990. Time course of inhibition of caffeine elimination in response to the oral depot contraceptive agent Deposiston. Hormonal contraceptives and caffeine elimination. *Zentralblatt fur Gynakologie,* 113(6): 298.

9. Ortweiler, W., Simon, H.U., Splinter, F.K., Peiker, G., Siegert, C. and Traeger, A., 1984. Determination of caffeine and metamizole elimination in pregnancy and after delivery as an in vivo method for characterization of various cytochrome p-450 dependent biotransformation reactions. *Biomedica biochimica acta,* 44(7-8): 1189-1192.

10. Mazaleuskaya, L.L., Sangkuhl, K., Thorn, C.F., FitzGerald, G.A., Altman, R.B. and Klein, T.E., 2015. PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. *Pharmacogenetics and genomics,* 25(8): 416.

11. Wang, S.F., Xie, F. and Hu, R.F., 2007. Carbon-coated nickel magnetic nanoparticles modified electrodes as a sensor for determination of acetaminophen. *Sensors and Actuators B: Chemical*, 123(1): 495.

12. Yilmaz, S., Bas, Z., Sadikoglu, M., Yagmur, S. and Saglikoglu, G., 2016. Sensitive Voltammetric Determination of Paracetamol on Poly (4-Aminobenzene Sulfonic Acid) Modified Glassy Carbon Electrode. *International Journal of Electrochemical Science,* 11(7): 6244.

13. Afrasiabi, M., Kianipour, S., Babaei, A., Nasimi, A.A. and Shabanian, M., 2016. A new sensor based on glassy carbon electrode modified with nanocomposite for simultaneous determination of acetaminophen, ascorbic acid and uric acid. *Journal of Saudi Chemical Society*, 20: 480.

14. Goyal, R.N. and Bishnoi, S., 2012. Surface modification in electroanalysis: past, present and future. *Indian Journal of Chemistry,* 51A: 205.

15. Lourenc, B.C., Medeiros, R.A., Romeu,C. R., Mazo, L.H., Fatibello-Filho, O., 2009. Simultaneous voltammetric determination of paracetamol and caffeine in pharmaceutical formulations using a boron-doped diamond electrode. *Talanta*, 78: 748–750

16. Khoshhesab, Z.M., 2015. Simultaneous electrochemical determination of acetaminophen, caffeine and ascorbic acid using a new electrochemical sensor based on CuO–graphene nanocomposite. *RSC Advances*, 5(115): 95140-95143.

17. Silva, T.A., Zanin, H., Corat, E.J. and Fatibello-Filho, O., 2017. Simultaneous Voltammetric Determination of Paracetamol, Codeine and Caffeine on Diamond-like Carbon Porous Electrodes. *Electroanalysis*, 29(3): 907-910.

18. Kassa, H., Geto, A. and Admassie, S., 2013. Voltammetric determination of nicotine in cigarette tobacco at electrochemically activated glassy carbon electrode. *Bulletin of the Chemical Society of Ethiopia*, 27(3): 321.

19. Fagan, D.T., Ing-feng, H.U. and Kuwana, T., 1995. Vacuum heat treatment for activation of glassy carbon electrodes. *Analytical chemistry*, 57(14): 2759-2763.

20. Qiao, J.X., Luo, H.Q. and Li, N.B., 2008. Electrochemical behavior of uric acid and epinephrine at an electrochemically activated glassy carbon electrode. *Colloids and Surfaces B: Biointerfaces*, 62(1): 31&32.

21. Zen, J.M., Senthil Kumar, A. and Tsai, D.M., 2003. Recent updates of chemically modified electrodes in analytical chemistry. *Electroanalysis*, 15(13): 1077.

22. Ranganathan, S., Kuo, T.C. and McCreery, R.L., 1999. Facile preparation of active glassy carbon electrodes with activated carbon and organic solvents. *Analytical chemistry*, 71(16): 3575.

23. Chitravathi, S. and Munichandraiah, N., 2016. Voltammetric determination of paracetamol, tramadol and caffeine using poly (Nile blue) modified glassy carbon electrode. *Journal of Electroanalytical Chemistry*, 764: 94.

24. Kuskur, C.M., Swamy, K.B. and Jayadevappa, H., 2015. Electrochemical investigation of paracetamol at poly (glycine) modified carbon paste electrode: a voltametric study. *Journal of Analytical &Bioanalytical Techniques,* 6(4):2155.

25. Guo, D.J. and Jin, M., 2013. Electrocatalytic oxidation and the mechanism of dopamine on a MWNT-modified glassy carbon electrode. *Russian Journal of Electrochemistry,* 49(2): 201.

26. Câmpean, A., Tertiş, M. and Săndulescu, R., 2011. Voltammetric determination of some alkaloids and other compounds in pharmaceuticals and urine using an electrochemically activated glassy carbon electrode. *Central European Journal of Chemistry,* 9(4): 688

27. Ellis, F., 2002. Paracetamol: a curriculum resource. *Royal Society of Chemistry*.10.

28. Zajac, M.A., Zakrzewski, A.G., Kowal, M.G. and Narayan, S., 2003. A novel method of caffeine synthesis from uracil. *Synthetic communications*, 33(19): 3292-3293.

29. González-Calderón, D., González-Romero, C., González-González, C.A. and Fuentes-Benítes, A., 2015. Synthesis of caffeine from theobromine: Bringing back an old experiment in a new setting. *Educación Química*, 26(1): 9-10.

30. Harvey, D., 2000**.** Modern analytical chemistry. New York: McGraw-Hill.381: Pp 667.

31. Aoki, K. and Osteryoung, J., 1980. Theory of differential pulse polarography at expanding or stationary planar electrodes for quasi-reversible or totally irreversible reactions. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, 110(1-3): 19-21.

32. Kounaves, S.P., 1997. Voltammetric techniques, Handbook of Instrumental Techniques for *Anal. Chem*, 709: Pp 709-711

33. Brajter-Toth, A., 2003. Electroanalytical Methods: Guide to Experiments and Applications Edited by Fritz Scholz (E.-M.-Arndt-Universität Greifswald). Springer-Verlag: Berlin, Heidelberg, New York. 2002. xxii+ 332 pp. $69.95. ISBN 3-540-42229-3. Pp 25-26

34. Bard, A. J., Faulkner, L.R., 2001. Electrochemical Methods: Fundamental and Applications, 2nd edition, John Wiley, New York. Pp 234- 236.

35. Atta, N.F., Galal, A., Abu-Attia, F.M. and Azab, S.M., 2011. Simultaneous determination of paracetamol and neurotransmitters in biological fluids using a carbon paste sensor modified with gold nanoparticles. *Journal of Materials Chemistry*, 21(34): 13015-13017.

36. Nouws, H.P., Delerue-Matos, C., Barros, A.A. and Rodrigues, J.A., 2006. Electroanalytical determination of paroxetine in pharmaceuticals. *Journal of pharmaceutical and biomedical analysis*, 42(3):

37. Skoog, D.A. and West, D.M., 2002. Fundamentals of Analytical Chemistry, Eight Edition. United States of America: Nesbitt graphies. Pp 742-743.

38. Brycht, M., Skrzypek, S., Kaczmarska, K., Burnat, B., Leniart, A. and Gutowska, N., 2015. Square-wave voltammetric determination of fungicide fenfuram in real samples on bare boron-doped diamond electrode, and its corrosion properties on stainless steels used to produce agricultural tools. *Electrochimica Acta*, 169: 117-118

39. Abu-Qare, A.W. and Abou-Donia, M.B., 2001. A validated HPLC method for the determination of pyridostigmine bromide, acetaminophen, acetylsalicylic acid and caffeine in rat plasma and urine. *Journal of pharmaceutical and biomedical analysis*, 26(5): 939.

40. Naseri, A. and Majidi, M. R., 2011. Cheap and easy modification of glassy carbon electrode for voltammetric determination of dopamine in the presence of ascorbic acid. *Journal of Faculty of Pharmacy, Tehran University of Medical Sciences*,19(4): 270.

41. Tadesse, Y., Tadese, A., Saini, R.C. and Pal, R., 2013. Cyclic voltammetric investigation of caffeine at anthraquinone modified carbon paste electrode. *International Journal of Electrochemistry*, 2013: 4-5

42. Tefera, M., 2016. Chemically Modified Electrodes for the Electrochemical Determination of Selected Pesticides and Pharmaceuticals (Doctoral dissertation, Addis Ababa University). 134.

43. Xiong, X.Q., Huang, K.J., Xu, C.X., Jin, C.X. and Zhai, Q.G., 2013. Glassy carbon electrode modified with poly (taurine)/TiO2-graphene composite film for determination of acetaminophen and caffeine. *Chemical Industry and Chemical Engineering Quarterly*, 19(3): 364-365.